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OPTIMIZATION OF VIRAL RNA EXTRACTION METHODS FOR DENGUE VIRUS DETECTION IN FIELD COLLECTED *Aedes albopictus*

By

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DECLARATION

I hereby declare that this thesis is my original work and has not been submitted previously or currently for any other degree at UiTM or any other institutions.

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ABSTRACT

OPTIMIZATION OF VIRAL RNA EXTRACTION METHODS FOR DENGUE VIRUS DETECTION IN FIELD COLLECTED *Aedes albopictus*

Dengue transmission has become a global threat and a health concern in tropical and sub-tropical countries. *Aedes albopictus* (Skuse), has high infestation rates in both urban and suburban areas of human settlement and is endemic in many Asian countries including Malaysia. The durability of the dengue virus (DENV) has been correlated with the occurrence of transovarial transmission via its vector. The detection of transovarial DENV transmission is complex and requires high quality and concentration of viral RNA. There are many methods of extraction available in literature, some superior than others. The purpose of this study was to determine the most optimum extraction method for DENV detection and to detect transovarial transmission of DENV using real time PCR (qRT-PCR) in field collected *Ae. albopictus*. A laboratory strain from USM was used to optimize the method of viral RNA extraction. *Ae. Albopictus* samples were collected from non-hotspot areas at Kolej Angsana, A5, UiTM Puncak Alam. The mosquitos were reared and hatched until adulthood in the laboratory. Both male and female *Ae. Albopictus* were selected, pooled and homogenized for RNA extraction. Modified PBS extraction with QIAamp viral RNA mini kit extraction protocol was then chosen for the sample extraction for this study. The outcome of this study has shown that the most suitable extraction method for DENV detection is by the use of modified PBS extraction method with QIAamp viral mini kit. In addition, no DENV was detected in all samples tested despite having optimized the best method for viral RNA extraction. This could be a result of the field work being collected during non-outbreak seasons, especially at non-hotspot areas. Further study is warranted to confirm these findings using a different locality.

Keywords: *Ae. Albopictus*, DENV, qRT-PCR, transovarial

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Dengue transmission has become the global threat that has been a health concern in tropical and sub-tropical countries (Gulati & Maheshwari, 2007). The severity of the infection can lead to dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) which can cause fatal. According to WHO 2014, *Aedes albopictus* is another *Aedes spp.* which has eventually acclimatize to the rural area of living, which includes urban and suburban area of human settlement.

The origin of this species of mosquito are said to be from the South East Asia of Indian Ocean and Western Pacific however, earlier in 20th century it has diversify their population across Pacific islands (Gratz, 2004). WHO 2014, has also stated that although *Aedes aegypti* is the main vector for dengue transmission, *Aedes albopictus* has been proven to become the most eligible vector for the arbovirus in certain area of settlement. To date, the control measures are limited as there are no licensed and reliable vaccines to be used to overcome this disease (Thomas, 2011).

The durability of the dengue virus has also been correlated with the occurrence of transovarial transmission of it where it was significant in most endemic country worldwide (Lee & Rohani, 2005). The theory is supported by a study by (Lequime & Lambrechts, 2014) where the horizontal transmission of arthropod-borne virus is mostly demonstrated by invertebrate vectors that feed on blood. This incident enables the virus to withstand in variety condition of environment such as surviving during cold weather or